Novel aspects regarding mechanisms and consequences of albuminuria

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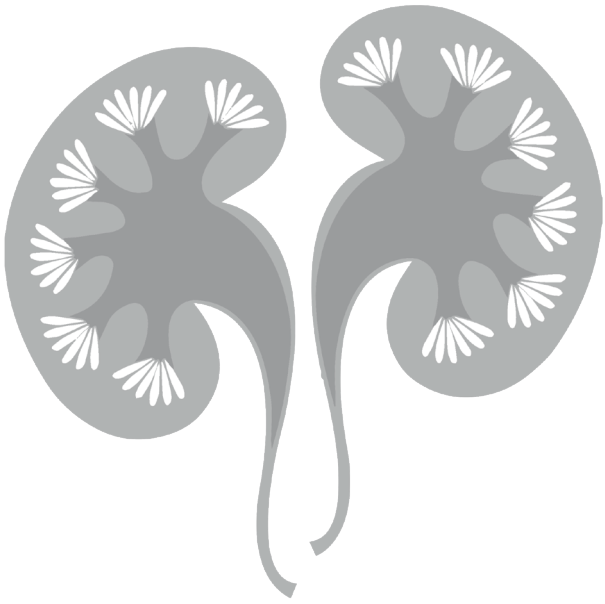

Chapter 7

The association of albuminuria with tubular reabsorption of uric acid. Results from a general population cohort

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Abstract

Background

Elevated albuminuria as well as an increased serum uric acid concentration is associated with poor cardiovascular outcome. We questioned whether these two variables (albuminuria and serum uric concentration) may be interrelated via tubular uric acid reabsorption

Methods

Included were 7,688 participants of the PREVEND Study, an observational, general population based cohort study. Linear regression analyses were used to test associations of baseline albuminuria with baseline serum uric acid concentration and tubular uric acid reabsorption (calculated as (100-fractional uric acid excretion) %). Cox regression analyses were used to study the association of baseline serum uric acid and albuminuria with incident cardiovascular morbidity and mortality.

Results

In cross-sectional analyses albuminuria was positively associated with serum uric acid concentration, both crude and after adjustment for potential confounders (both $p<0.001$). Albuminuria was found to be positively associated with tubular uric acid reabsorption, again both crude and after adjustment for potential confounders (both $p<0.001$). In longitudinal analyses during a median follow-up of 10.5 years, 702 cardiovascular events occurred. After adjustment for cardiovascular risk factors both albuminuria and serum uric acid were associated with incident cardiovascular events (Hazard Ratios 1.09 (1.03-1.17), $p=0.01$ and 1.19 (1.09-1.30), $p<0.001$, respectively). A significant interaction between these variables was present ($p<0.001$), consistent with high serum uric acid being less predictive for cardiovascular morbidity and mortality in the presence of high albuminuria and vice versa.

Conclusions

Albuminuria is strongly associated with tubular uric acid reabsorption, and consequently with serum uric acid concentration. This phenomenon may explain in part why albuminuria is associated with cardiovascular outcome.

Introduction

Uric acid is the final oxidation product of purine catabolism in humans. For decades it has been hypothesized that the antioxidant properties of uric acid might be protective against aging, oxidative stress, and oxidative cell injury (1). However, recent epidemiological and clinical evidences suggest that hyperuricaemia might be a risk factor for cardiovascular disease, where enhanced oxidative stress plays an important pathophysiological role (1,2). The apparent paradox between protective and toxic effects is supported by clinical evidences that antioxidant compounds may become pro-oxidant compounds in certain situations, particularly when they are present in blood at supranormal levels (1). In line, several epidemiological studies have reported an association between elevated serum uric acid concentration and increased albuminuria. (3-7) In general this association has been explained as serum uric acid causing endothelial dysfunction, which is reflected by an increase in albuminuria.(8)

Uric acid is the end product of purine metabolism. The mechanism of renal uric acid handling is complex. In blood, urate is only minimally bound to proteins. Therefore almost 100% is freely filtered at the glomerulus into the renal tubule. (9,10) In the proximal tubule sequentially almost complete reabsorption, secretion and post-secretory reabsorption take place. These processes are mediated by various urate transporters at the apical and basolateral membrane of proximal epithelial tubular cells.(10) In concert they cause that in healthy individuals reabsorption of urate is relatively constant at about 95%.(10)

Like serum uric acid also albuminuria has been found to be associated with increased risk for cardiovascular disease. The amount of albumin that is lost in urine is the resultant of the amount that is filtered by the glomerulus and the amount that escapes degradation or reabsorption by the proximal tubule. Interestingly, recent studies have shown that proximal epithelial tubular cells that are exposed to albumin differentially up- and down regulate genes encoding for membrane transporters.(11-13) Others have shown that non-albumin compounds found in urine of proteinuric patients, such as plasmin, influence the activity of membrane transporters. (14)

Given these data we questioned whether albuminuria may increase serum uric acid by influencing the activity of tubular transport mechanisms. If such an interaction between albuminuria and serum uric acid concentration were to exist, it may be anticipated that the associations of albuminuria and serum uric acid with cardiovascular outcome will show an interaction, i.e. that in subjects with higher albuminuria the association of serum uric acid with cardiovascular outcome is less strong.

We investigated these hypotheses using data of a large scale, observational cohort study in which data on serum and urine uric acid concentration, as well as albuminuria and cardiovascular outcome are available.

Patients and Methods

Study design and population

This study was conducted using data obtained in subjects who participate in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, which started in 1997. This prospective cohort study investigates the natural course of albuminuria and its relation to renal and cardiovascular disease. Details of the study protocol have been published elsewhere.[15, 16] In summary, all inhabitants of the city of Groningen aged 28–75 years were sent a questionnaire on demographics, disease history, smoking habits, use of medication and a vial to collect a first-morning-void urine sample. Of these subjects, 40,856 responded (47.8%). From these subjects, the PREVEND cohort was selected with the aim to create a cohort enriched for subjects with higher albuminuria. After exclusion of patients with type 1 diabetes mellitus (defined as requiring the use of insulin) and pregnant females (defined by self report), all subjects with a urinary albumin concentration of >10 mg/L (7,768) were invited, of which 6,000 participated. Furthermore, a randomly selected control group with a urinary albumin concentration of <10 mg/L (3,394) was invited, of which 2,592 participated. These 8,592 subjects constitute the actual PREVEND cohort and were studied in more detail.

For the current study participants were excluded with missing data on serum or urine uric acid concentration (N=210 and 54, respectively), serum or urine creatinine concentration (N=34 and 33, respectively), urinary albumin excretion (N=2), and participants who were using drugs influencing uric acid metabolism (diuretics, allopurinol and benzpromaron, N=349). Furthermore, participants with outlier values for serum uric acid (N=18) and tubular reabsorption (N=6), and assumed errors in 24h-urine collection were excluded (N=198), leaving 7,688 subjects for the present study. The PREVEND study was approved by the medical ethics committee of our institution and conducted in accordance with the International Conference of Harmonization Good Clinical Practice Guidelines and adheres to the ethical principles that have their origin in the Declaration of Helsinki.

Measurements and Definitions

At the baseline visit participants filled in a questionnaire, anthropometrical measurements were performed, and fasting blood samples were taken. In addition, subjects collected urine for two consecutive periods of 24 hours. Blood pressure was measured in supine position with an

automatic device (Dinamap XL Model 9300; Johnson-Johnson Medical, Tampa, FL). Blood pressure is given as the mean of the last two recordings.

Concentrations of total cholesterol and plasma glucose were measured using standard methods. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, New York, USA). Urinary albumin concentration was measured by nephelometry with a threshold of 2.3 mg/L and intra- and interassay coefficients of variation of 2.2 and 2.6%, respectively (BNII; Dade Behring Diagnostic, Marburg, Germany). Urinary albumin excretion is given as the mean of the two 24h urine collections. Serum concentrations of uric acid were measured with The Merck Mega clinical chemistry analyzer with the uricase PAP (peroxidase-aminophenazone) method, with an intra- and interassay coefficient of variation of 1.1% and 1.3%, respectively. Urinary uric acid has been measured with the Merck Mega, with and intra-assay and interassay coefficient of variation 0.9% and 1.4%, respectively.

Participants were considered to be smoking when according to the questionnaire they had smoked in the year prior to the baseline screening, and as using alcohol when according to the questionnaire they had used at least 1-4 drinks a month in the year prior to the baseline screening. Cardiovascular disease history was defined as self reported myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft or cerebrovascular accident. Information on drug use in general was obtained from the questionnaire, and on specific drug use from the Inter-Action Data-Base (IADB), which comprises pharmacy-dispensing data of community pharmacists located in the northern regions of the Netherlands. These pharmacies provide the IADB database a complete listing of patient-specific dispensed drugs. (17) Hypertension was defined as systolic blood pressure of ≥140 mmHg, a diastolic blood pressure ≥90 mmHg or use of antihypertensive medication according to self report or to pharmacy data. Hyperlipidemia was defined as cholesterol level >5.0 mmol/L in participants with a cardiovascular disease history, a cholesterol level of >6.5 mmol/L when such a history was absent or use of lipid lowering drugs according to self report or to pharmacy data. Diabetes was defined as a fasting glucose level of >7.0 mmol/L, a non-fasting glucose level of >11.1 mmol/L or use of anti-diabetic medication according to self report or to pharmacy data. eGFR was estimated using the CKD-EPI equation and body surface area (BSA) unadjusted. (18) Body surface area was calculated with the DuBois formula.(19) Body mass index was calculated as the ratio between weight and the square of height (weight/height^2), with obesity defined as a body mass index above 30 kg/m^2.

Increased serum uric acid is defined as a serum level of more than 0.35 mmol/L for females and more than 0.45 mmol/L for males as criteria for increased serum urate levels, similar to

prior reports (6,20). Uric acid excretion is defined as 24h-urinary uric acid excretion. Fractional excretion of uric acid (%) was calculated as ([urine uric acid] * [serum creatinine]) / ([urine creatinine] * [serum uric acid] *100), and tubular uric acid reabsorption (%) as 100-(fractional uric acid excretion).

Outlier values were calculated for serum uric acid and tubular reabsorption of uric acid, and were defined as a value which falls more than 1.5 times the interquartile range above the third quartile or below the first quartile.(21) Errors in 24h-urine collections were defined as the upper and lower 2.5% of the difference between expected and actually measured 24h-urine volume. The expected 24h-urine volume was calculated by comparing creatinine clearance estimated by the Cockgroft-Gault (CG) formula[22] and actual creatinine clearance (24h-urine volume = (CG * [serum creatinine]) / [urinary creatinine]).

Cardiovascular outcome

For cardiovascular outcome we used the incidence of the combined outcome of cardiovascular morbidity and mortality. Date and cause of death were obtained by record linkage with the Dutch Central Bureau of Statistics. Information on hospitalization for cardiovascular morbidity was obtained from PRISMANT, the Dutch national registry of hospital discharge diagnoses. All data were coded according to the International Classification of Diseases, 9th revision and the classification of interventions. For this study, cardiovascular events were defined according the Major Adverse Cardiovascular Events (MACE) criteria as acute myocardial infarction (ICD-code 410), acute and subacute ischaemic heart disease (code 411), subarachnoid haemorrhage (code 430), intracerebral haemorrhage (code 431), other intracranial haemorrhage (code 432), occlusion or stenosis of the precerebral (code 433) or cerebral arteries (code 434), coronary artery bypass grafting or percutaneous transluminal angioplasty, and other vascular interventions such as percutaneous trans- luminal angioplasty or bypass grafting of aorta peripheral vessels.

Statistical analysis

Continuous data are reported as means ± standard deviation (SD). In case of non-parametric data distribution medians with interquartile range (IQR) are presented. Differences between groups for continuous data were tested by Student's t-test or a Mann-Whitney U test in case of non-parametric data distribution. Differences between groups for proportions were tested with a Chi-square test.

We first performed linear regression analyses to test associations of baseline albuminuria with baseline serum uric acid or tubular uric acid reabsorption (cross-sectional analyses), in univariable and in multivariable linear regression analyses. Because urinary albumin

excretion was not normally distributed, this variable was logarithmically transformed to meet the assumptions for linear regression analyses. These regression analyses were performed crudely, as well as adjusted for potential confounders that are known to influence renal uric acid handling. For these analyses the standardized beta coefficients are given in the tables. These refer to how many standard deviations a dependent variable will change per standard deviation increase in the predictor variable. Standardization of the coefficient is usually done to answer the question which of the independent variables has a greater effect on the dependent variable in a multiple regression analysis, when the variables are measured in different units.

We next performed Cox-regression analyses to investigate the association of albuminuria and serum uric acid with cardiovascular morbidity and mortality during follow-up (longitudinal analyses). These analyses were performed univariate as well as multivariate, adjusting for traditional cardiovascular risk factors and potential confounders. In these models the Hazard Ratio of continuous variables is expressed per standard deviation of each variable, which allows direct comparison of the strength of associations of individual variables in the multivariate model. Furthermore, albuminuria and serum uric acid were simultaneously entered in the multivariable model. For Cox-regression analyses survival time was defined as the time between the date of urine collection of the participant and the date of first cardiovascular event or January 1st, 2009 (end-of-follow-up). Subjects were censored in case they died or moved to an unknown destination. In addition, it was investigated whether there was an interaction between urinary albumin excretion and serum uric acid in their association with cardiovascular morbidity and mortality by adding the interaction term albuminuria * serum uric acid to the full adjusted model containing also both variables as single terms.

Figures 1 and 2 were generated from results of linear regression analyses. In the multivariable analysis the Hazard Ratios are adjusted for age, gender, use of alcohol, BMI, eGFR, and 24h urinary excretion of uric acid.

We performed several sensitivity analyses: we first repeated our analyses using design based regression analyses, in which the fact that by design the PREVEND cohort is enriched for subjects with higher albuminuria levels was taken into account. The advantage is that results can be extrapolated to the general population. Second, all the linear and Cox regression analyses were performed using urinary albumin concentration instead of 24-hour urinary albumin excretion, to investigate whether incorrect 24h urine collection may have influenced our results. Third, fractional sodium excretion and hs-CRP were added to the multivariable model investigating the associations of albuminuria with serum uric acid concentration and tubular reabsorption of uric acid, because these variables may be additional confounders in the association of albuminuria with serum uric acid concentration and uric acid reabsorption.

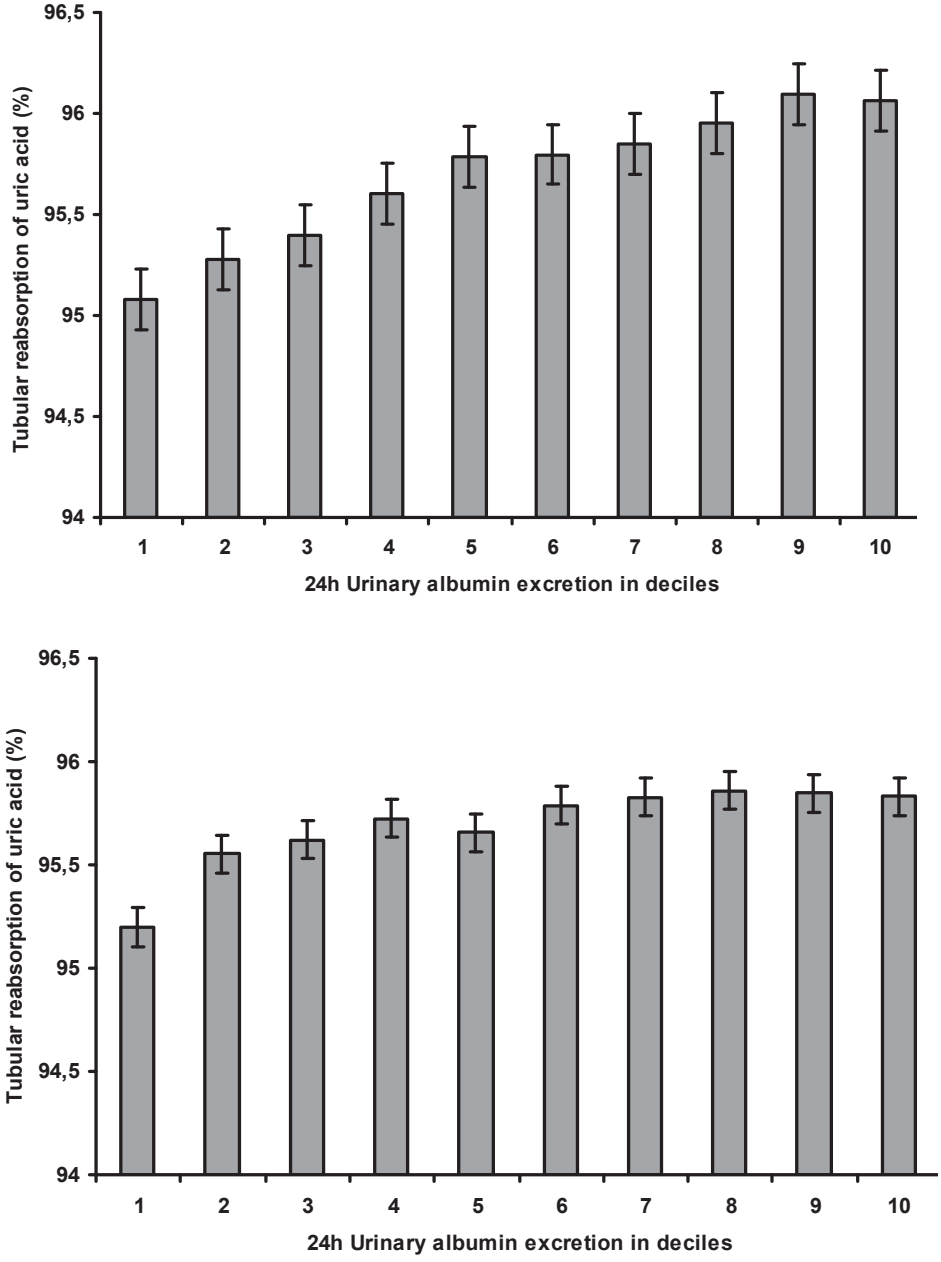

Figure 1. Association between urinary albumin excretion and tubular reabsorption of uric acid (mean and standard deviation), with the population stratified in deciles of urinary albumin excretion. Upper panel shows crude analyses; lower panel shows the mean values adjusted for age, gender, use of alcohol, BMI, eGFR, and 24h urinary excretion of uric acid.

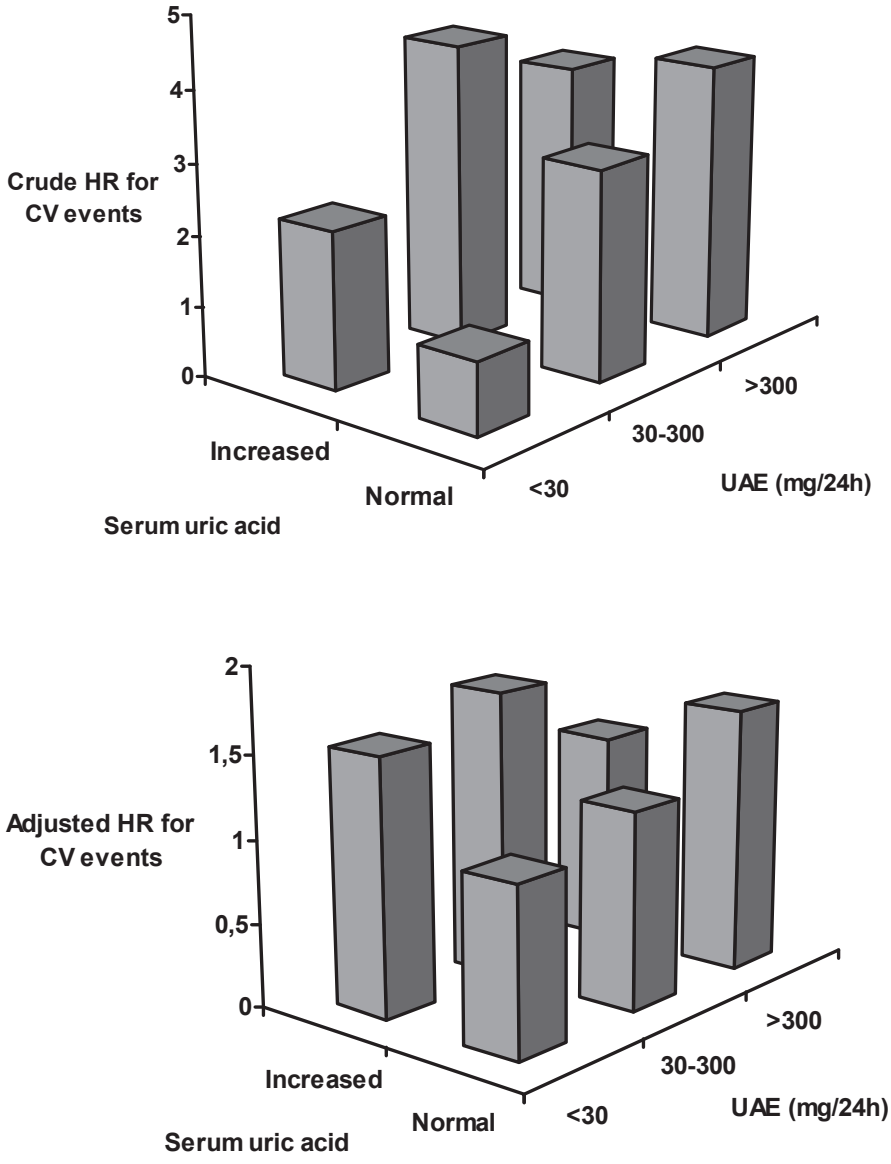

Figure 2. Association between urinary albumin excretion, serum uric acid and relative risk for cardiovascular morbidity and mortality. The upper panel shows crude Hazard Ratio's, the lower panel Hazard Ratio's adjusted for cardiovascular risk factors (age, gender, smoking, history of cardiovascular disease, obesity, diabetes, hypertension and hypercholesterolemia). Reference category (HR=1) was chosen to be normal serum uric acid (<0.45 and <0.35 mmol/L in males and females, respectively) and normoalbuminuria (<30 mg/24h).

All calculations were performed with SPSS version 18.0 software, and for all analyses a two-sided p-value <0.05 was considered to indicate statistical significance.

Results

Mean age of the 7,688 study participants was 48.6±12.4 years. Approximately half of the participants were of male gender. Mean serum uric acid was 0.30±0.08 mmol/L. Mean urinary uric acid excretion was 1.7±0.7 mmol/24h, tubular uric acid reabsorption 95.7±2.1% and median urinary albumin excretion 9.3 [6.3-17.5] mg/24h (Table 1). When dividing the study population into subjects with normo- versus increased albuminuria (>30 mg/24h) it shows that 1,124 participants had increased albuminuria (median urinary albumin excretion 60.8 [40.0-115.2] mg/24h). These participants are significantly older, more often of male gender, have a lower eGFR, a higher serum uric acid concentration, and a higher tubular uric acid reabsorption, while uric acid excretion was similar (Table 1).

Baseline associations between albuminuria, serum uric acid, urinary uric acid excretion and tubular reabsorption of uric acid

Albuminuria was positively associated with serum uric acid concentration, both crude (model 1) and in models that adjusted for potential confounders, including age and gender (model 2), alcohol consumption, BMI, eGFR and 24h-urinary uric acid excretion (model 3) and even after additional adjustment for tubular reabsorption of uric acid (model 4) (Table 2, all p<0.001), although a significant attenuation of effect was noted moving from the unadjusted to the fully adjusted models.

When studied separately, albuminuria was significantly positively associated with tubular uric acid reabsorption, again crude (model 1), as well as in models that adjusted for potential confounders (models 2 through 4) (Table 3), the higher albuminuria the higher tubular reabsorption of uric acid. This association is visualised in Figure 1, with the study population subdivided according to deciles of 24h-albuminuria. The upper panel shows the crude association of albuminuria with tubular uric acid reabsorption, and the lower panel the association when adjusted for the covariates of the multivariable regression model.

Associations of albuminuria and serum uric acid with cardiovascular events during follow-up

During a median follow-up of 10.5 years (0.03-11.3 years), 702 cardiovascular events occurred. In separate models serum uric acid and albuminuria were both significantly associated with cardiovascular morbidity and mortality, both crude (Hazard Ratio (HR) per standard deviation

Table 1. Baseline characteristics of the total study cohort and when stratified according to albuminuria status.

	Total cohort N=7,688	UAE <30 mg/24h N=6,564	UAE >30 mg/24h N=1,124	p-value
Age (yr)	48.6 ±12.4	47.5 ±12.1	55.4 ±12.4	<0.001
Male gender (%)	50.5	48.2	63.9	<0.001
Body mass index (kg/m^2)	26.0 ±4.2	25.7 ±4.0	27.9 ±4.8	<0.001
Obesity (%)	14.9	12.8	26.9	<0.001
Smoking (%)	38.1	37.7	40.3	0.10
Cardiovascular history (%)	4.9	3.7	11.7	<0.001
Use of alcohol (%)	75.0	75.5	71.8	0.01
Systolic blood pressure (mmHg)	128.4 ±19.9	125.9 ±18.1	142.9 ±23.4	<0.001
Diastolic blood pressure (mmHg)	73.8 ±9.7	72.7 ±9.1	79.9 ±11.0	<0.001
Antihypertensive medication (%)	12.2	9.8	25.8	<0.001
Hypertension (%)	31.0	25.7	62.0	<0.001
Serum glucose (mmol/L)	4.8 ±1.1	4.8 ±0.9	5.4 ±1.9	<0.001
Glucose lowering medication (%)	1.2	0.8	3.2	<0.001
Diabetes (%)	3.3	2.1	9.9	<0.001
Serum cholesterol (mmol/L)	5.6 ±1.1	5.6 ±1.1	5.9 ±1.1	<0.001
Lipid lowering medication (%)	3.6	2.9	8.1	<0.001
Hyperlipidemia (%)	27.2	24.9	40.5	<0.001
Serum creatinine (µmol/L)	83.5 ±14.9	82.5 ±13.2	89.4 ±21.4	<0.001
Serum uric acid (mmol/L)	0.30 ±0.08	0.30 ±0.07	0.33 ±0.08	<0.001
eGFR (mL/min)	93.9 ±19.7	94.4 ±19.1	90.2 ±22.2	<0.001
Urinary albumin excretion (mg/24h)	9.3 [6.3-17.5]	8.2 [6.0-12.4]	60.8 [40.0-115.2]	<0.001
Urinary uric acid excretion (mmol/24h)	1.7 ±0.7	1.7 ±0.7	1.7 ±0.8	0.91
Tubular reabsorption of uric acid (%)	95.7 ±2.1	95.6 ±2.1	96.1 ±2.1	<0.001

Abbreviations: eGFR, estimated glomerular filtration rate.

(SD) 1.66 (1.55-1.77) and 1.53 (1.45-1.61), respectively, both p<0.001), and after adjustment for traditional cardiovascular risk factors (HR per SD 1.21 (1.10-1.32), p<0.001 and 1.11 (1.04-1.18), p=0.002, respectively). When albuminuria and serum uric acid concentration were entered simultaneously in the multivariable adjusted model, both were significantly associated

Table 2. Association of urinary albumin excretion with serum uric acid (dependent variable), both crude and after adjustment for confounders

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln albuminuria (mg/24h)	0.23	<0.001	0.12	<0.001	0.07	<0.001	0.03	0.001
Age (yrs)			0.12	<0.001	-0.12	<0.001	-0.06	<0.001
Gender (female vs. male)			-0.51	<0.001	-0.61	<0.001	-0.36	<0.001
Use of alcohol (yes vs. no)					0.09	<0.001	0.06	<0.001
BMI (kg/m^2)					0.38	<0.001	0.21	<0.001
eGFR (ml/min)					-0.29	<0.001	-0.39	<0.001
Uric acid excretion (mmol/24h)					-0.03	0.001	0.43	<0.001
Tubular reabsorption of uric acid (%)							0.69	<0.001

Abbreviations: ln: natural log-transformed; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-equation). Standardized beta coefficients refer to how many standard deviations a dependent variable will change per standard deviation increase in the predictor variable. This allows a comparison which of the independent variables has a greater effect on the dependent variable in multiple regression analysis, when the variables are measured in different units.

with cardiovascular outcome (standardised HR for albuminuria 1.09 (1.03-1.17), p=0.01 and for serum uric acid HR 1.19 (1.09-1.30), p<0.001) (Table 4). In the multivariate model in which urinary albumin excretion and serum uric acid concentration were simultaneously entered, it appeared that there was a significant interaction between these variables (p<0.001), consistent with high serum uric acid being less strongly associated with cardiovascular morbidity and mortality in the presence of high albuminuria and vice versa.

This association of baseline albuminuria and serum uric acid concentration with cardiovascular outcome during follow-up is visualised in figure 2. Both higher albuminuria and higher serum uric acid concentration are associated with a higher relative risk for cardiovascular morbidity and mortality, and a negative interaction is observed: in participants with higher albuminuria the risk for cardiovascular morbidity and mortality associated with a high serum uric acid concentration is less strong, both crude (upper panel) as well as adjusted for cardiovascular risk factors (lower panel).

Table 3. Association of urinary albumin excretion with tubular reabsorption of uric acid (dependent variable), both crude and after adjustment for confounders

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln albuminuria (mg/24h)	0.12	<0.001	0.07	<0.001	0.03	0.01	0.06	<0.001
Age (yrs)			-0.03	0.004	-0.03	0.03	-0.08	<0.001
Gender (female vs. male)			-0.36	<0.001	-0.31	<0.001	-0.36	<0.001
Use of alcohol (yes vs. no)					0.01	0.56	0.05	<0.001
BMI (kg/m^2)					0.25	<0.001	0.25	<0.001
eGFR (ml/min)					0.11	<0.001	0.14	<0.001
Uric acid excretion (mmol/24h)							-0.66	<0.001

Abbreviations: ln: natural log-transformed; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-equation). Standardized beta coefficients refer to how many standard deviations a dependent variable will change per standard deviation increase in the predictor variable. This allows a comparison which of the independent variables has a greater effect on the dependent variable in multiple regression analysis, when the variables are measured in different units.

Table 4. Association of urinary albumin excretion and serum uric acid with risk for cardiovascular morbidity and mortality, adjusted for cardiovascular risk factors. Results are standardized, indicating that Hazard Ratios (HRs) are given per standard deviation (SD) of the variable under investigation.

	HR (95% CI)	p-value
Ln albuminuria (mg/24h) per SD	1.09 (1.03-1.17)	0.01
Serum uric acid (mmol/L) per SD	1.19 (1.09-1.30)	<0.001
Age (yrs) per SD	2.23 (2.00-2.50)	<0.001
Gender (F)	0.50 (0.41-0.61)	<0.001
Use of alcohol (%)	0.71 (0.60-0.84)	<0.001
Obesity (%)	1.13 (0.94-1.37)	0.20
History of CVD (%)	1.67 (1.33-2.09)	<0.001
Smoking (%)	2.00 (1.71-2.34)	<0.001
Diabetes Mellitus (%)	1.19 (0.89-1.59)	0.24
Hypertension (%)	1.55 (1.29-1.86)	<0.001
Hypercholesterolemia (%)	1.45 (1.22-1.72)	<0.001
eGFR (mL/min) per SD	1.04 (0.95-1.15)	0.41

Abbreviations: ln: natural log-transformed; SD: standard deviation; CVD: cardiovascular disease; eGFR: estimated glomerular filtration rate (CKD EPI-equation)

Sensitivity analyses

Several sensitivity analyses were performed. First, when design-based analyses were performed, accounting for the fact the PREVEND cohort by design is enriched for participants with higher levels of albuminuria, all aforementioned analyses rendered essentially similar results (data not shown). Second, we performed all analyses using urinary albumin concentration instead of urinary albumin excretion. Again essentially similar results were obtained. Urinary albumin concentration was significantly associated with serum uric acid concentration (Supplementary Table 1) and tubular uric acid reabsorption (Supplementary Table 2), both crude as well as in multivariable models. Urinary albumin concentration and serum uric acid concentration were both significantly associated with cardiovascular outcome in the multivariable model (HR 1.09 (1.03-1.18), p=0.01 and HR 1.19 (1.09-1.30), p<0.001, respectively) (Supplementary Table 3), and the negative interaction between these two variables in their association with cardiovascular outcome was again significant (p<0.001). Third, because it has been suggested that albuminuria and serum uric acid concentration may also be dependent on sodium balance, serum albumin and systemic inflammation, we added fractional sodium excretion (mean 0.69 ±0.2%), serum albumin (mean 45.8 ±2.7 g/L) and high-sensitivity CRP (median 1.2, IQR 0.5-2.9 mg/L) to the multivariable model. This influenced only slightly the association between albuminuria and serum uric acid concentration (fully adjusted model standardized beta 0.02, p=0.01, Supplementary Table 4) and the association between albuminuria and tubular reabsorption of uric acid (fully adjusted model standardized beta 0.05, p<0.001, Supplementary Table 5).

Discussion

In the present study, that uses data obtained in a large, general population based cohort study, we show that albuminuria is positively associated with tubular uric acid reabsorption and serum uric acid concentration. Additionally we observed that there is an interaction between albuminuria and serum uric acid in their association with cardiovascular morbidity and mortality.

The last decades several studies have been published that showed an association between albuminuria and serum uric acid.(3-7,23-28) This association has been observed in subjects with heart failure(26), diabetes mellitus (5,6,23,28-29) and hypertension (27,30), and even in subjects participating in general population studies.(3,4) Most of these studies were cross-sectional in design. Only a limited number of studies investigated whether a high baseline serum uric acid predicts development of microalbuminuria. These studies showed contradictory results; with some describing that serum uric acid was independently associated with *de novo*

microalbuminuria. In other studies, however, this association was only found in crude analyses, but not in analyses adjusting for confounders. In yet another study even the crude association was absent. Despite these contradictory longitudinal results, the cross-sectional associations between albuminuria and serum uric acid concentration have generally been interpreted as uric acid causing endothelial dysfunction, which is reflected by an increase in albuminuria.

In the present study we found a positive association of albuminuria with tubular uric acid reabsorption, independent of potential confounders a.o. hs-CRP.(31) It may well be that the expression of genes encoding for tubular uric acid transporters is specifically up- or downregulated by albumin, or non-albumin compounds found in urine of albuminuric subjects. Such a phenomenon has been observed for other, non-urate membrane transporters in cultured tubular proximal epithelial cells (11-14). Of note, our data suggest that nothing much changes for tubular reabsorption of uric acid in the upper deciles of urinary albumin excretion. This could mean that, after a sensitive range at lower values of albuminuria, stimulation of reabsorption or perhaps reabsorption as such is at a maximum. Another mechanism could theoretically be that the association we found is in fact mediated by sodium reabsorption, since sodium depleted subjects have an increased tubular reabsorption of sodium and at the same time increased tubular reabsorption of uric acid.(32) In our opinion this mechanism is less likely to play a role, because we found that the association between albuminuria and tubular uric acid reabsorption was independent of fractional sodium excretion. Furthermore, it is well established that not sodium depleted, but on the contrary sodium repleted subjects have higher albuminuria.(33) To unravel the exact mechanism of the association that we found, dedicated, in-depth experimental studies will be necessary that investigate the role of the various renal uric acid transporters in high albuminuria states. Our results form a rationale to start such research. The association of albuminuria and increased tubular urate reabsorption may well explain the association between albuminuria and serum uric acid concentration, as an increased reabsorption will result in a rise in serum uric acid concentration. The opposite, i.e. an increase in serum urate concentration, will not result in an increased, but more likely a decreased tubular urate reabsorption. We therefore, hypothesize that albuminuria may be causal in the association between albuminuria and serum uric acid concentration.

In this study we corroborate that both albuminuria and serum uric acid concentration are independently associated with risk for cardiovascular morbidity and mortality.(8,34) High serum uric acid may cause endothelial dysfunction and therefore vascular damage (8,35,36), although antioxidant properties have also been attributed to serum urate, but especially in the lower concentration range.(1,37) The mechanism why albuminuria is associated with cardiovascular outcome is still unknown. In this respect only few studies have investigated the predictive role

of both variables entered simultaneously into a multivariable model, but these studies did not investigate cardiovascular outcome.(29,38) We found that both variables were significantly associated with risk for cardiovascular outcome, independent of traditional cardiovascular risk factors and independent of each other. In addition, we observed a significant interaction between these two variables in their association with cardiovascular outcome, which has not been investigated before and may therefore have remained unreported to date. This latter observation suggests that both variables are associated with outcome via a (partially) overlapping mechanism, which fits our hypothesis that higher albuminuria may cause an increase in serum uric acid concentration by increasing renal tubular uric acid reabsorption. We would like to emphasize that this mechanism is unlikely to be the only explanation why albuminuria is associated with risk for cardiovascular morbidity and mortality, because albuminuria is still associated with risk even when adjusted for serum uric acid concentration.

Our study has limitations that need to be mentioned. First, our results are obtained in a predominantly Caucasian population, and consequently cannot directly be extrapolated to other ethnic groups. Second, as the intra-individual level of urinary albumin excretion is subject to variation, e.g. due to physical activity and inflammatory diseases, misclassification may have occurred. Special care was therefore taken to assess urinary albumin excretion as precise as possible. Participants were advised not to collect urine in case of fever and to refrain from intensive physical activity during the urine collection period. Furthermore, participants with likely errors in their 24h-urine sampling were excluded. Most important, misclassification is expected to bias results towards the null-hypothesis (i.e. no association), whereas in fact a significant association of albuminuria with serum uric acid concentration and tubular uric acid reabsorption was found. Third, our results regarding the associations of albuminuria and tubular urate reabsorption and serum urate are obtained in a cross-sectional analysis, which precludes firm conclusions on cause-effect relationships.

Strengths of our study are that our data were obtained in a relatively large scale epidemiological study that was specifically designed to study the course and consequences of albuminuria. As such the PREVEND cohort is enriched for higher levels of albuminuria, making it particularly suited for studies investigating the association of albuminuria with other variables. Of note, even when adjusted for study design essentially similar results were obtained, making our data robust. Furthermore, albuminuria was assessed immediately in two fresh 24h urine collections, whereas in most epidemiological studies albuminuria is assessed in one random spot urine sample after prolonged frozen storage, which is known to be subject to more variability.(39,40) Lastly, in our participants extensive information is available on a large number of covariates, including medication use, which allows adjustment for confounders in multivariable analyses.

In conclusion, our study showed a strong association between albuminuria and uric acid reabsorption. This relationship could be due to albuminuria enhancing proximal tubular urate reabsorption. Controlling for serum uric acid levels strongly decreased the predictive value of albuminuria with regard to cardiovascular events. This phenomenon may explain in part why albuminuria is associated with cardiovascular outcome.

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Disclosures

None.

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Supplementary Table 1. Association between urinary albumin concentration (instead of 24h urinary albumin excretion) and serum uric acid (dependent variable), crude as well as adjusted for confounders

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln Urinary albumin concentration (mg/L)	0.22	<0.001	0.11	<0.001	0.05	<0.001	0.02	0.03
Age (yrs)			0.13	<0.001	-0.11	<0.001	-0.06	<0.001
Gender (female vs. male)			-0.51	<0.001	-0.61	<0.001	-0.36	<0.001
Use of alcohol (yes vs. no)					0.09	<0.001	0.06	<0.001
BMI (kg/m^2)					0.38	<0.001	0.21	<0.001
eGFR (mL/min)					-0.29	<0.001	-0.39	<0.001
Urinary uric acid excretion (mmol/24h)					-0.02	0.03	0.43	<0.001
Tubular reabsorption of uric acid (%)							0.69	<0.001

Abbreviations: ln: natural log-transformed; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-equation)

Supplementary Table 2. Association between urinary albumin concentration (instead of 24h urinary albumin excretion) and tubular reabsorption of uric acid (dependent variable), crude as well as adjusted for confounders

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln Urinary albumin concentration (mg/L)	0.24	<0.001	0.19	<0.001	0.15	<0.001	0.05	<0.001
Age (yrs)			-0.05	<0.001	-0.05	<0.001	-0.08	<0.001
Gender (female vs. male)			-0.34	<0.001	-0.29	<0.001	-0.35	<0.001
Use of alcohol (yes vs. no)					0.01	0.22	0.05	<0.001
BMI (kg/m^2)					0.24	<0.001	0.25	<0.001
eGFR (mL/min)					0.11	<0.001	0.14	<0.001
Urinary uric acid excretion (mmol/24h)							-0.65	<0.001

Abbreviations: ln: log-transformed; ACR: albumin-creatinine-ratio; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-formula) rate (CKD EPI-equation)

Supplementary Table 3. Associations of urinary albumin concentration (instead of 24h urinary albumin excretion) and serum uric acid concentration with cardiovascular morbidity and mortality, adjusted for cardiovascular

	HR (95% CI)	p-value
Ln Urinary albumin concentration (mg/L) per SD	1.09 (1.03-1.18)	0.005
Serum uric acid (mmol/L) per SD	1.19 (1.09-1.30)	<0.001
Age (yrs) per SD	2.23 (1.99-2.50)	<0.001
Gender (female vs. male)	0.50 (0.41-0.61)	<0.001
Use of alcohol (%)	0.72 (0.61-0.85)	<0.001
Obesity (%)	1.15 (0.95-1.39)	0.16
History of CVD (%)	1.68 (1.33-2.11)	<0.001
Smoking (%)	2.00 (1.71-2.34)	<0.001
Diabetes Mellitus (%)	1.20 (0.90-1.61)	0.21
Hypertension (%)	1.55 (1.29-1.86)	<0.001
Hypercholesterolemia (%)	1.44 (1.21-1.71)	<0.001
eGFR (mL/min) per SD	1.04 (0.94-1.15)	0.42

Abbreviations: ln: log-transformed; SD: standard deviation; CVD: cardiovascular disease; eGFR: estimated glomerular filtration rate (CKD EPI-equation)

Supplementary Table 4. Association between urinary albumin excretion and serum uric acid (dependent variable), crude as well as adjusted for confounders, among which hs-CRP and fractional sodium excretion.

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln albuminuria (mg/24h)	0.23	<0.001	0.12	<0.001	0.07	<0.001	0.02	0.01
Age (yrs)			0.12	<0.001	-0.12	<0.001	-0.03	0.003
Gender (female vs. male)			-0.51	<0.001	-0.61	<0.001	-0.31	<0.001
Use of alcohol (yes vs. no)					0.09	<0.001	0.06	<0.001
BMI (kg/m^2)					0.38	<0.001	0.15	<0.001
eGFR (ml/min)					-0.29	<0.001	-0.34	<0.001
Uric acid excretion (mmol/24h)					-0.03	0.001	0.42	<0.001
Tubular reabsorption of uric acid (%)							0.73	<0.001
Ln hsCRP (mg/L)							0.06	<0.001
Serum albumin (g/L)							0.06	<0.001
Fractional sodium excretion (%)							0.14	<0.001

Abbreviations: ln: natural log-transformed; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-equation); hsCRP: high sensitive CRP

Supplementary Table 5. Association between urinary albumin excretion and tubular reabsorption of uric acid (dependent variable), crude as well as adjusted for confounders, among which hs-CRP and fractional sodium excretion.

	Model 1		Model 2		Model 3		Model 4	
	St B	p-value	St B	p-value	St B	p-value	St B	p-value
Ln albuminuria (mg/24h)	0.12	<0.001	0.07	<0.001	0.03	0.01	0.05	<0.001
Age (yrs)			-0.03	0.004	-0.03	0.03	-0.09	<0.001
Gender (female vs. male)			-0.36	<0.001	-0.31	<0.001	-0.38	<0.001
Use of alcohol (yes vs. no)					0.01	0.56	0.04	<0.001
BMI (kg/m^2)					0.25	<0.001	0.27	<0.001
eGFR (ml/min)					0.11	<0.001	0.09	<0.001
Uric acid excretion (mmol/24h)							-0.63	<0.001
Ln hsCRP (mg/L)							0.04	<0.001
Serum albumin (g/L)							0.06	<0.001
Fractional sodium excretion (%)							-0.15	<0.001

Abbreviations: ln: natural log-transformed; BMI: body mass index; eGFR: estimated glomerular filtration rate (CKD EPI-equation); hsCRP: high sensitive CRP